14
TRANSCRIPTION FACTOR HES1 MODULATES OSTEOARTHRITIS DEVELOPMENT IN COOPERATION WITH CAMKII

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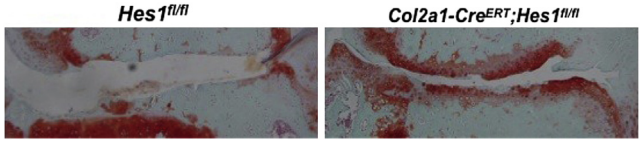
Purpose: We recently reported that the RBPj-dependent Notch signaling in chondrocytes modulates endochondral ossification and osteoarthritis (OA) development. Since this signal was shown to be mediated through induction of the target gene Hes1 in chondrocytes, the present study investigated the role of Hes1 and the underlying mechanism during OA development.
Methods: We generated tissue-specific knockout mice of Hes1 by mating *Sox9-Cre* knock-in mice or tamoxifen-inducible *Col2a1-Cre* transgenic mice (*Col2a1-Cre^{ERT}*) with mice homozygous for a floxed Hes1 allele (*Hes1$^{fl/fl}$*). To analyze the role Hes1 in articular cartilage after maturation, we injected tamoxifen into 7-week-old *Col2a1-Cre^{ER-T};Hes1$^{fl/fl}$* mice, and created a surgically induced OA model by resecting the medial collateral ligament and the medial meniscus in the knee joints one week after the injection. The OA severity was quantified by the OARSI histopathology grade 8 weeks after the surgery. We examined transcriptional regulation by chromatin immunoprecipitation (ChIP) sequencing using human chondrogenic SW1353 cells transfected with the FLAG-tagged Hes1 construct, and confirmed the transactivation by luciferase assay using HeLa cells transfected with the reporter construct containing a promoter fragment of the marker genes. For expression analyses, we performed immunofluorescence and real-time RT-PCR. For protein-protein interaction analyses, we performed co-immunoprecipitation (Co-IP) assay in SW1353 cells. For functional analyses, we used lentiviral doxycycline-inducible expression vectors in mouse chondrogenic ATDC5 cells and examined the expression of target genes by real-time RT-PCR.
Results: Although the *Sox9-Cre;Hes1$^{fl/fl}$* mice died in the perinatal period, the embryos exhibited normal sketetal growth. However, the OA development was prevented in the *Col2a1-Cre^{ERT};Hes1$^{fl/fl}$* knee joints more than in the control *Hes1$^{fl/fl}$* joints. OARSI grading confirmed that conditional knockout of Hes1 caused significant resistance to cartilage degradation. Immunofluorescence revealed that Mmp13 and Adamts5 expressions were suppressed in the articular cartilage of *Col2a1-Cre^{ERT}* mice. ChIP sequencing showed the highest binding of Hes1 in intron 4 of *MMP13* and in intron 7 of *ADAMTS5*, and the luciferase assay confirmed that Hes1 stimulated the enhancer activities of these elements. Although Hes1 is generally known to be a transcriptional repressor, the present data suggest that Hes1 directly enhances *Mmp13* and *Adamts5* expressions. To reveal the molecular mechanism underlying transcriptional induction of these catabolic factors by Hes1, we focused on calcium/calmodulin-dependent protein kinase II (CaMKII), which is the only factor known to change Hes1 from transcriptional repressor to transcriptional activator by phosphorylating it. Among subtypes of CaMKII, the δ form was most highly expressed in mouse articular chondrocytes. Immunofluorescence of the mouse experimental model revealed that both Hes1 and active CaMKII were increased in articular cartilage during OA progression. Co-IP confirmed the binding of Hes1 and CaMKIIδ proteins. When both these proteins were lentivirally co-overexpressed in ATDC5 cells, *Mmp13* and *Adamts5* expressions were induced more highly than a single overexpression of each gene. Furthermore, a mutagenesis of Hes1 in the serine residue of CaMKII phosphorylation site caused a loss of its ability to induce *Mmp13* and *Adamts5*.
Conclusions: Hes1 modulates OA development through the transactivation of Mmp13 and Adamts5 through the phosphorylation by CaMKIIδ. Notch/Rbpj and Hes1 pathways in cooperation with CaMKIIδ may be possible therapeutic targets of OA.



15
RECEIVING OPERATING CHARACTERISTICS ANALYSIS OF OUTCOMES OF TOTAL JOINT REPLACEMENT FOR OSTEOARTHRITIS

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Purpose: Persistent pain after joint replacement is a considerable problem affecting between 7 and 20% of total knee replacement (TKR) patients and 2-8% of total hip replacement (THR) patients. The aim of the present study was to assess the factors that contribute to patient satisfaction and mobility post joint replacement.
Methods: 735 knee and hip osteoarthritis patients were recruited from orthopaedic clinics 1 year post total joint replacement and a research nurse assessed their level of mobility, satisfaction with their surgery, medical history, pain intensity, PainDETECT questionnaire scores, quality of life and quality of sleep. Catastrophizing and illness behavior were also available for a subset of patients.
Results: Among the 308 patients who had undergone THR, 98% were very satisfied of the outcome of their surgery, 94% reported a significant pain improvement and 83% an improvement in their ability to walk post THR, 3.9% had undergone a revision surgery. This compared to 75% satisfaction in the 383 post TKR cases, 3.1% of whom had a revision surgery, 85% reported a pain improvement and 72% an improvement in mobility. Among patients who had undergone both TKR and THR procedures satisfaction was 84%, 90% reported a substantial pain improvement even though the rate of revision surgery has much higher (13.6%)than among TKR only or THR only cases. The factors that contributed to lack of satisfaction post surgery in THR cases were the WOMAC pain score (OR=.25 95% CI 1.05-1.48 per Likert scale unit $p<0.012$) younger age (OR=0.92 95%CI 0.86-0.99 per year $p<0.018$) and presence of revision surgery (OR=8.13 95%CI 1.4-46 $p<0.018$). Among post-TKR patients the strongest factors contributing to lack of satisfaction were the WOMAC pain score (OR=1.15 95%CI 1.06-1.24 per Likert scale unit), poor sleep (OR=1.25 95%CI 1.04-1.49 in a scale 0-10) and presence of possible neuropathic pain (as defined by the PainDETECT questionnaire) (OR=1.90 95%CI 1.03-3.49). Among patients with both TKR and THR procedures the only significant factor contributing to satisfaction was the WOMAC stiffness score (OR=3.39 95%CI 1.21-9.48 per Likert scale unit). An analysis in a subset of 163 post-TKR cases on which catastrophizing measures were available revealed that, although a high catastrophizing score is indeed associated with satisfaction, this association was entirely mediated by pain and dropped from the model after adjusting for WOMAC pain scores. Using a receiver operating characteristics (ROC) analysis we found that among TKR cases, age, sex and BMI provide no useful information on patient satisfaction (area under the curve(AUC)=0.496). On the other hand the presence of possible neuropathic pain (NP) alone achieved an AUC=0.643 [95%CI 0.58 - 0.705] on patient